

## Review

## Fractal analysis in a systems biology approach to cancer

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## ABSTRACT

Cancer is a highly complex disease due to the disruption of tissue architecture. Thus, tissues, and not individual cells, are the proper level of observation for the study of carcinogenesis. This paradigm shift from a reductionist approach to a systems biology approach is long overdue. Indeed, cell phenotypes are emergent modes arising through collective non-linear interactions among different cellular and microenvironmental components, generally described by “phase space diagrams”, where stable states (attractors) are embedded into a landscape model. Within this framework, cell states and cell transitions are generally conceived as mainly specified by gene-regulatory networks. However, the system's dynamics is not reducible to the integrated functioning of the genome–proteome network alone; the epithelia–stroma interacting system must be taken into consideration in order to give a more comprehensive picture. Given that cell shape represents the spatial geometric configuration acquired as a result of the integrated set of cellular and environmental cues, we posit that fractal-shape parameters represent “omics” descriptors of the epithelium–stroma system. Within this framework, function appears to follow form, and not the other way around.

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## 1. Introduction

Hornberg et al. [1], have stated that “cancer is [...] a systems biology disease. Indeed, progress in cancer research toward cancer therapy may develop faster if cancer is not researched only in terms of molecular biology, but rather in terms of systems biology”. A systems biology (SB) approach requires a “systematic integration of data” that is provided by high-throughput techniques at a rate inconceivable until few years ago [2]. Under this heading, adopting an “SB framework” is nothing more than relying on an efficient database of the already known and stored immense *corpus* of molecular details about cancer biology. Admittedly, the relevance of this *corpus* for carcinogenesis is rather controversial, to say the least. An SB approach is not merely a matter of computational tools; it requires, instead, a radical shift from an old into a *new paradigm* [3]. This transition emerges “from the ashes of genetic reductionism” [4], fostered by the awareness that “the key obstacle to future medicine is the conflict between the reality of complexity and a reductionistic approach” [5]. In fact, even partisans of the “reductionistic approach”, now recognize that not only the classic carcinogenic paradigm, but also the scientific methodology behind

it, “are no more maintainable” [6,7]. Herein, we sketch a general frame for a systemic cancer appreciation, first, by giving a reliable and *operational* definition of “system,” and second, by reaching in depth into the relevant features of ‘cancer as a system’. Finally, we highlight the relevance of the shape of cells and tissues as studied by fractal analysis in the construction of a reliable phase space for cancer development.

## 2. The system

Modern biology does not explicitly takes into account the problem of many levels of observation, thus ignoring the possibility of considering a biological entity as a “system”. Moreover, after the initial widespread, positive reception to the Central Dogma of Biology, the paradigm of gene-centered biology has been “illegitimately extended as a paradigm of life” [8], thus yielding confusion between different hierarchical organization levels: “Now we are mixing our levels in biology and it does not work” [8].

Cancer research has focused on molecular entities (genes, enzymatic reactions, intracellular pathways), under the implicit assumption that molecules have their proper autonomy and behave like a “biological system”, i.e., a group of entities that work together to perform a certain task. However, “a system is not just an assembly of genes and proteins [and] its properties cannot be fully understood merely by drawing diagrams of their interconnections”

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[8]. From a thermodynamic point of view, any part of the universe could be legitimately considered a “system”, provided its borders can be defined in a sufficiently reliable way. Nevertheless, when referring to a “system” in biology, focusing only on thermodynamics can be misleading. Thermodynamics typically refer to a macroscopic view of the system at hand defined by macro-variables (i.e., temperature, pressure, volume) that allow for a reliable prediction of relevant system’s features. This reliability comes from the statistics over a huge number of particles without the need of explicitly taking into consideration the correlative structure generated by the among particles, i.e., without any particular concern on the ‘shape’ of the system at hand.

In biological systems, shape plays a crucial role and thus, it becomes imperative to focus at the so called ‘mesoscopic’ level of observation [9], where the interaction among elements of the system take place and where shape is generated. In architecture, the mesoscopic level is located at the level of the arch, while a brick is microscopic and the entire building is the macrolevel. By comparing the form of the arches, one can discriminate between a Romanesque and a Gothic cathedral, while this task is impossible to accomplish by either an analysis of the bricks or of the general plan of the construction. Therefore, for a proper system-level understanding – the approach advocated in systems biology – a shift in our notion of “what to look for” is required.

Reductionism derived macroscopic behaviour from microscopic details “obeying a theory of everything” [10]. As pointed out by Strohmman “it is the *mesoscopic organization* of matter (living or dead) that harbours as yet undiscovered principles lying behind emergent features” [11]. Within this framework, the genome does not “explain anything”, even if one were to believe that it represents the ‘digital core of information’ [12] on which higher-level system properties are thought to be “mechanistically linked” [13]. Confusion results from conflating the two gene-based properties, i.e., protein-coding functions and involvement in hereditary character transmission [14]. Nevertheless, this does not actually imply the existence of a ‘privileged’ level of causality: there is no one-to-one correspondence between genes or proteins and higher-level biological functions. As outlined by Noble, it is a prejudice what inclines us to give a causal priority to a lower-level (molecular) and “the concept of level in biology is itself metaphorical” [15]. Clearly, this latter quote provides a different definition of genome that no longer can be interpreted as the “molecular hardware” of a “genetic program” [16]. The newly formulated “middle way” searches for rules of self organization appropriate to the mesoscopic domain, in order to find useful approximations to how things work. Such approximations have led to major insights in physics and even in biology [16].

This mesoscopic level must include cells and their microenvironment (stromal cells and extracellular matrix components) where carcinogenesis takes place [17]. In addition to the upward causation prevalent in reductionism, downward causation should also be included. The mesoscopic level is where organizational principles act on the elementary biological units that will become altered, or constrained, by both their mutual interaction and the interaction with the surrounding environment. In this way and in this place is where general organization behaviour emerges and where we expect to meet the elusive concept of complexity.

### 3. Complex systems

According to Prigogine [18], a complex system should (1) possess information; (2) be neither strictly ordered (like a crystal) nor fully disordered (like a gas); (3) be thermodynamically open, meaning that it deals with non-linear dynamics; (4) display emergent collective properties (different from those of the

component sub-systems); and (5) have a *history*, meaning that the present behaviour of the system is in part determined by its *past behaviour*. The information content of a system is based on Shannon’s mathematical theory of information [19]. This theory is based on the statistical probability of occurrence of the different discrete instances (states) of a system. In order to apply such a paradigm we need to know *a priori* the entire set of possible states; this is the reason why the mathematical theory of information was successfully applied to biopolymers, where the instantaneous states of the system are fully known (nucleotides for DNA, aminoacid residues for protein sequences). By analogy, total information in the living cell was often identified mistakenly with genetic information; ‘genome information content’ could hardly be representative of the overall cell information content. For instance, differences between genome sequences of humans and mice are not correlated with the differences of form and function between them [20]. Moreover, there are many examples in which no or little correlation exists between genetic and morphological complexity, pointing out that information relevant for function could not be located solely at the genomic level [21]. This means that, broadly speaking, the informational content of a living cell is something different than that of its genome [22]. A telling example of this mistaken view is exemplified by the multiple dog phenotypes that share a mostly identical genome [23].

In complex systems, information is expressed as *negative entropy*. Attempts to give an operational definition of negative entropy and anti-entropy [24] as a measure of complexity were made. Moreover, because classical Shannon’s communication theory is not ideally suited for describing what is meant by ‘biological network information’, numerous papers evaluate the information content of several kinds of networks [25,26] with the aim of obtaining a compelling definition of ‘complexity’. Indeed, while complexity measures abound, their relationship to biology are not always clear. Moreover, some theoretical approaches are hardly of practical interest, given that parameters and variables thought to measure complexity are difficult to compute and to be translated into biologically meaningful features [27]. In addition, a complex system can display several kinds of complexity that cannot be expressed by the classic and generic notion of entropy [28].

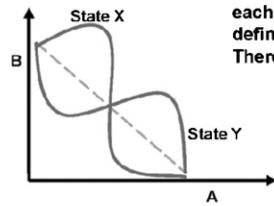
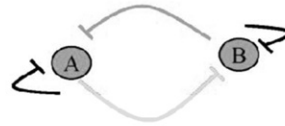
A suitable, but not exhaustive, conceptualisation of complexity should be addressed in terms of the required information for system description, as proposed by Chaitin [29]. Accordingly, a system could be considered “maximally complex when the rate of change of the irreducible amount of information required describing that system with respect to some parameter or parameter set is an *extremum*. This means that the absolute value of the slope of the amount of irreducible information required to describe the system versus some parameters reaches a maximum [...] when the system is maximally complex” [30]. Such a definition fits well with one of the measures of complexity we shall consider, i.e., the fractal dimension of cell shape, while at the same time allows to directly tackle the problem of multiple hierarchical levels and non-linearities.

### 4. Attractors and phase-space diagram

Regulation of cell functions can be thought of as *physically and topologically structured* molecular interactions and physical forces located at different hierarchical levels. In dissipative systems, such as living organisms, the overall system behaves according to a non-linear dynamics [31]. The dynamics of such a system is the concerted change in the levels of  $x_i(t)$  [the value of the node  $i$ ] for all the nodes  $i$  of the network (a metabolomic or genomic network) and can be represented by the  $N$ -dimensional *state vector*:  $S(t) = [x_1(t), x_2(t), \dots, x_N(t)]$ . While the topology represents

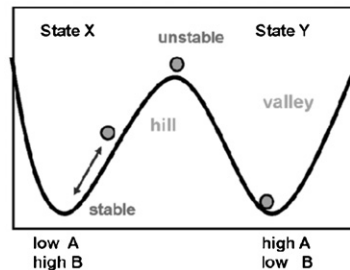
A Example: a small circuit with  $N = 2$ 

Two proteins that are mutually inhibitory and promote their own decay. For a wide range of kinetic parameter this topology give rise to *bistability*.



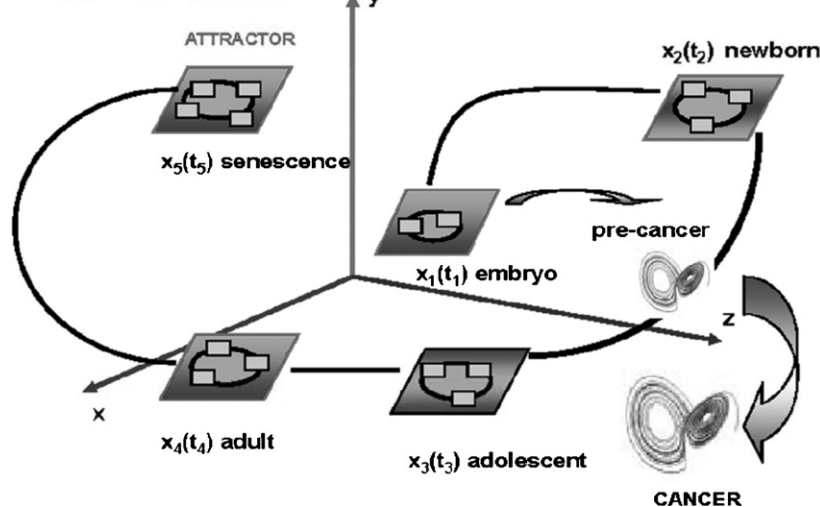
A two-dimensional state phase, with 2 stable states (X,Y): each dot represents a state of the circuit  $S(t) = [x_A(t), x_B(t)]$  defined by the activity values of the 2 nodes A and B. There are 2 stable states X and Y

An intuitive projection of the state space emphasizing the bistability of the system, with attractors (valleys) and meta-stable or unstable conditions (hill)



## B

$Y, X, Z = \text{state parameter}$



**Fig. 1.** Attractors and phase-space diagram. (A) A chemical reaction illustrating the phenomenon of bistability. The reaction is represented in terms of motion in a double-well potential. (B) The state vector  $S_{xy}(t)$  identify a specific phenotype, representing a stationary state: the trajectories emanating from the neighborhood are “attracted”. This stable attractor state is robust to many perturbations. Attractors are self-organizing structures and can “capture” the stability of gene expression profiles associated with cell fates. Transitions from one phenotype to another can be triggered by both internal and external regulatory signals that essentially “reset”  $S(t)$  by displacing the network state to another place in the state space. In the entire phenotypic state space there exist regions with attractors that encode the embryonic cell phenotypes not present in the mature organism and that generally are “not accessible” for the adult cells. The embryonic attractors may be far away in the state space from adult attractors, but they are likely to be near to cancer-attractor states.

the network by a graph, for a network with  $N$  nodes, the dynamics can be better described in the *Phase Space* (with  $N$ -dimension or degree of freedom) in which  $S(t)$  changes its position along the time coordinate  $t$  as defined by its components  $[x_1(t), x_2(t), \dots, x_N(t)]$ . The mathematical formalism of the phase space diagram for dissipative systems is described by the theory of dynamical non-equilibrium systems [32]. As proposed by Kauffman [33], the phase space describes a landscape characterized by attractors (“valleys”) – surrounded by basins – separated by “hills”: the difference in the behavioral potential between cells lies in their *position* on the landscape and the associated accessibility to attractors (Fig. 1). In this view, cell fate regulation is based on selection between pre-existing, limited, intrinsically robust fates. It must be stressed that the special state vector  $S^*(t)$  is a *stationary state* in which there is no net driving force. The trajectories emanating from

the neighbourhood are “attracted” and converge to  $S^*(t)$ . This stable attractor state is robust to many perturbations: “attractors” are, indeed, self-organizing structures and can “capture” gene expression profiles associated with cell fates [34].

A discrete finite number of attractor classes do exist. Generally, *strange attractors* (i.e., dynamics that do not follow a simple periodic trajectory) arise from non-linear dynamical systems. The *phenotypic traits* of the organism are embedded into the dynamic attractors of its underlying regulatory network [35,36]. Functional states depicted as attractors have been conceived as mainly specified by the gene-regulatory network [34]. However, “the stability of functional states clearly also depends on external cues” [37]. Thus, system’s dynamics in the phase space cannot be “reduced” either to a genetic wiring diagram or, even to the integrated functioning of a genome–proteome–metabolome network. Additional

influences – i.e., those of the intracellular topology that makes the chemical reactions of the “networks” possible, as well as those resulting from parenchyma–stroma interacting system – must be taken into consideration in order to give a more reliable definition of attractor. This revised conceptualisation of “attractors” could fit numerous observations on tissue dynamics, the existence of different dynamical regimens, as well as the transitions between them [38]. Moreover, this hierarchical organization creates downward causation [39] complementing the better known upward causation, and thereby shaping the complex behaviour of the system [40].

## 5. Shape as a measure of complexity

Like any other hierarchical construct, biological entities are thought to potentially adopt an undefined possibility of configurations (“forms”) ‘inside the realm’ of a common general frame. However, only a limited number of them is actually observed. In the case of protein structures, the number of folds is much lower than that expected when referring to the transfinite number of possible dispositions of  $N$  residues in space; different sequences may give rise to the same fold. This implies some sort of ‘energy minimization’ drastically constraining the number of allowable stable states [41], with the consequent onset of preferred stable states (attractors, in dynamic terms). Given that protein folding results from the non-linear interactions between internal (aminoacids sequence) as well as microenvironmental constraints, the resulting configuration can be considered as the integrated output of such complex and dynamic interplay taking place at the mesoscopic level.

Therefore, shape descriptors could be reliably used as overall indicators of macrostates. Indeed, cancer diagnosis as well as that of other diseases has been routinely made by looking at the cell and tissue shapes elicited by those pathological entities [42,43]. Moreover, quantitative measures of shape could be considered in depicting the phase space of choice, like tissue organization and structure. The shape of cells and the structures they form is the consequence of physical forces generated in the cytoskeleton as well as in the extracellular matrix. Shape, in reflecting cytoskeleton organization, is linked to the repertoire of metabolic events, which result from the right ordering in space of the enzymes catalysing specific pathways. Physical forces (like microgravity) induce dramatic changes in gene expression and alter cellular shape [44,45].

Despite limited knowledge about how living cells “sense” mechanical stresses, evidence suggests that relevant modifications in the expression of thousands of genes and in the activity of enzymatic reactions can be quickly elicited by modifications in cell shape. Changes in the balance of forces that are transmitted across trans-membrane adhesion receptors that link the cytoskeleton to other cells and to the extracellular matrix influence cell morphology and subsequently induce alterations in intracellular biochemical processes [46]. In this context, it is unlikely that the observed changes in cell phenotype and genome functions could be ascribed to a single (or a few) signalling pathways operating in isolation. Equally striking, the “dramatic” twisting of the tension-dependent form of (intracellular) architecture promptly leads to an overall modification in both the cell shape and of cytoskeleton-linked biochemical pathways [47]. Cells appear to be literally “hard-wired” so that they can filter the same set of inputs to produce different outputs, and this mechanism is largely controlled through physical distortion of plasma membrane adhesion receptors that transmit stress to the cytoskeleton. Thus, the switch between different cell fates could be considered dependent on cell-distortion [48]. Local geometric control of cell functions may hence

represent a fundamental mechanism for developmental regulation within the tissue microenvironment. Yet, a compelling theory explaining the link between shape and biochemical activity is still lacking. On the one hand, this is partly due to the limited knowledge about how biochemical reactions are associated to the cytoskeleton (i.e., the internal topology of structures-linked reactions), and, on the other, to a lack of a standardized and a widely accepted *measure* of cell shape complexity. A quantitative method that lends itself particularly useful for characterizing complex irregular structures is fractal analysis.

Although classical Euclidean geometry works well to describe properties of regular smooth-shaped objects such as circles or squares by using measures such as the length of the object’s perimeter, these Euclidean descriptions are inadequate for complex irregular-shaped objects that occur in nature (e.g., clouds, coastlines, and biological structures). These “non-Euclidean” objects are better described by fractal geometry, which has the ability to quantify the irregularity and complexity of objects with a measurable value called the fractal dimension. Fractal dimension differs from our intuitive notion of dimension in that it can be a non-integer value and the more irregular and complex an object is, the higher its fractal dimension [49].

Fractal analysis can lead to a remarkable improvement in both cyto-histological and radiographic diagnostic accuracy [50,51]. Applications of fractal measures to pathology and oncology [52] suggest that fractal analysis provides reliable and unsuspected information [53,54]. For instance, fractal analysis helped in discriminating benign from malignant neoplasms [55], low from high grade tumours [56]; furthermore, fractal studies elucidated some aspects of the complex interplay between cancer cells and stroma by suggesting that tumour vascular architecture is determined by heterogeneity in the cellular interaction with the extracellular matrix rather than by simple gradients of diffusible angiogenic factors [57]. Moreover, fractal analysis of the interface between cancer and normal tissues helps in understanding how cell detachment from the primary mass and infiltration into adjacent tissue occurs through a non-mutational mechanism [58,59].

A correlation between the fractal dimensions of the epithelia/stroma interface in the oral cavity with the evolving lesions (from dysplasia to invasive carcinoma) was linked to increases in the irregularity of the surface and of the fractal number (from 1.0 of normal epithelium to 1.62 for invasive tumour) [60]. Also, both the global and local fractal dimension of the epithelium–stroma interface increased from normal through pre-malignant to malignant oral epithelium [61] implying that the involvement of the epithelium–stroma interface is not merely a consequence of tumour development, but instead is an intrinsic feature of the carcinogenic process. On the contrary, P19 carcinoma cells undergoing differentiation when treated with all-trans retinoic acid, shows a significant reduction in their fractal dimension [62].

Collectively, these results highlight the relevance of shape–phenotype relationships that over three decades ago motivated Folkman and Moscona [63] to ask, “how important shape is” [60]? An answer to this question largely remains unknown. However, Ingber claimed that “the importance of cell shape appears to be that it represents a *visual manifestation of an underlying balance of mechanical forces that in turn convey critical regulatory information to the cell*” [64]. Thus, cell distortion influences cytoskeleton function and a cell’s adhesion to the ECM. Cell shape and cytoskeletal structure appear to be tightly coupled to cell proliferation [48]. In this way, tissue structure limits the constitutive ability of cells to proliferate [65]. Within this framework it seems as if “function follows form, and not the other way around” [66].



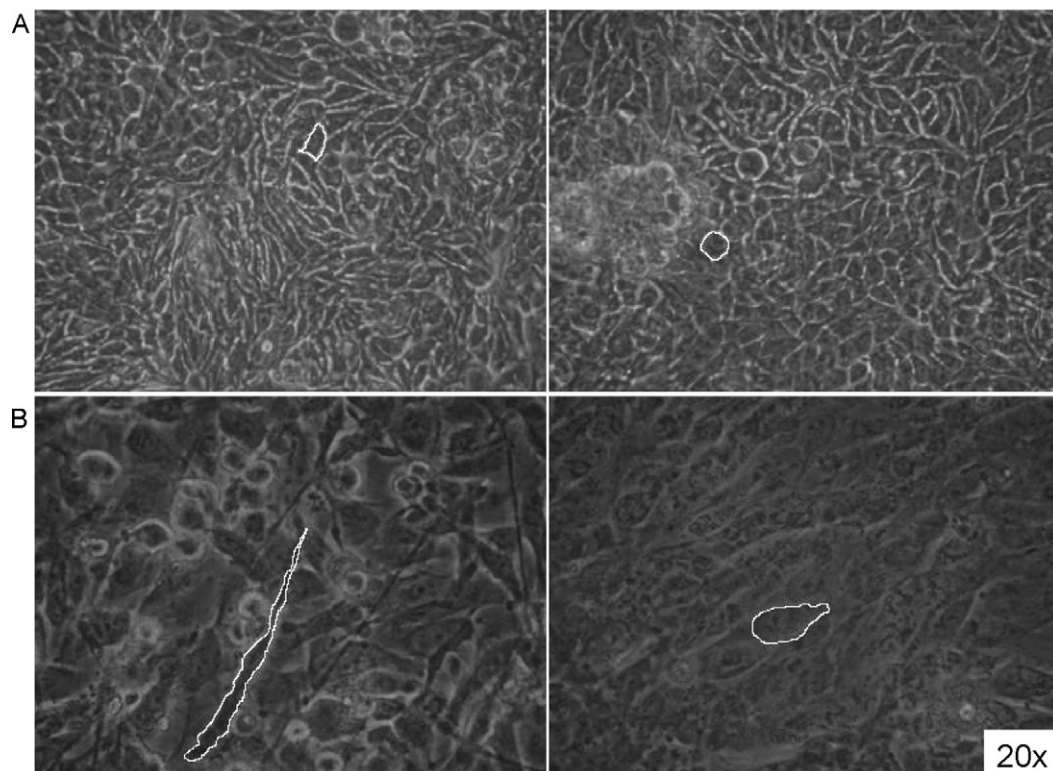
## 6. The biophysical bases of fractals

Mandelbrot [67] introduced the term ‘fractal’ (from the Latin *fractus*, meaning ‘broken’) to characterize spatial or temporal phenomena that are continuous but not differentiable. In fractal analysis, the Euclidean concept of ‘length’ is viewed as a *process*. This process is characterized by a constant parameter  $D$  known as the fractal (or fractional) dimension. The fractal dimension has a thermodynamic meaning, and can be viewed as an intensive measure [68] of the “overall” (morphologic) complexity [69]. Therefore, together with two or more independent variables, this would enable the construction of a diagram of phases, like that relying on temperature, pressure and volume for gas/liquid/solid phase-transitions. In fact, according to the Bendixon-Poincaré theorem, if a dynamic process possesses a limit cycle, (i.e., an attractor) then that attractor has fractal dimension. And *vice versa*, the existence of fractal dimension for a given dynamic process denotes that the process has been measured at its attractor [70]. In non-equilibrium systems, the fractal attractor is a common feature because of the dissipative character of these systems. The information dimension can then be used to determine the number of undamped dynamical variables which are active in the motion of the system; this means that dimension is something related to the number of degrees of freedom of the system. Although there may be many nominal degrees of freedom available, the physics of the system may organize the motion into *only a few* effective degrees of freedom. This collective behaviour is often termed self-organization and it arises in dissipative dynamic systems whose post-transient behaviour involves fewer degrees of freedom than are nominally available. The system is *attracted* to a lower-dimensional phase space, and the dimension of this reduced phase space represents the number of active degrees of freedom in the self-organized system. A similar trend can be observed during the shift from a morphotype to another in the course of the specialization/differentiation of a cell lineage. A cell

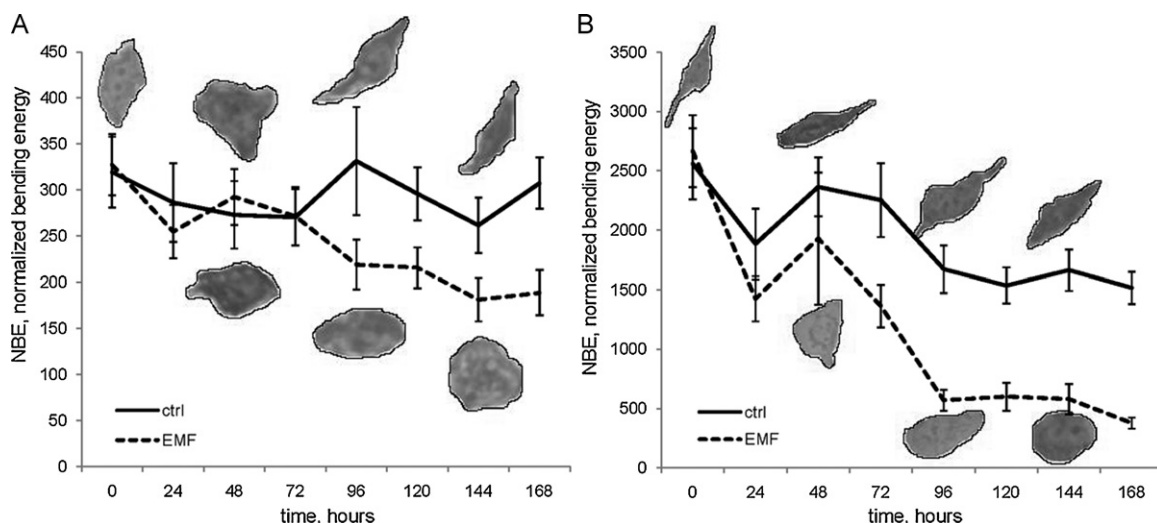
type proceeds through a discrete number of morphotypes along its specializing/differentiating pathway, and every morphotype could be considered as a *stable steady-state* [71]. In a similar way, morphologic characterization of a cell population by means of fractal analysis could provide at least an independent variable thought to be used to construct a (measurable) space phase of the evolving system, in order to characterize the attractors and the location of bifurcations.

## 7. Experimental results: how does a morphogenic field modify cell shape and tumour phenotype?

Mammary cancer epithelial cells form normal mammary ducts when transplanted into a normal mammary gland stroma [72]. Similarly, human cancer cells exposed to a microenvironment of normal embryonic cells revert toward a non-malignant phenotype [73–75]. Those cancer cells underwent a complex transition, involving morphologic as well as metabolic and functional modifications. Breast cancer cells growing in an *in culture* maternal-like morphogenic field (EMF) progressively undergo dramatic changes in both shape and metabolome reversion [76]. After 48 h, membrane profiles of breast cancer cells growing in EMF change evolving into a more rounded shape, loosing spindle and invasive protrusions (Fig. 2). Fractal analysis was carried out by calculating the Normalized Bending Energy (NBE) of cell membranes (Fig. 3). NBE characterizes shape by expressing the amount of energy needed to transform the specific shape under analysis into its lowest energy state (i.e., a circle) [77]. The “curvegram” obtained by using digital signal processing techniques gives a multi-scale representation of the curvature. As such, the bending energy provides a resource for translation and rotation-invariant shape classification, as well as a means of deriving quantitative information about the complexity of the shapes being investigated [78]. For biological shapes, NBE provides a particularly meaningful physical interpretation in terms



**Fig. 2.** Examples of breast cancer cell membrane segmentation using the GVF-Snake method. (A) MCF-7 cells in control (left) and after 96 hours of treatment with EMF (right). (B) MDA-MB-231 cells in control (left figure) and after 96 hours of treatment with EMF (right).



**Fig. 3.** Normalized Bending Energy of breast cancer cell membranes. (A) Normalized Bending Energy values for MCF-7 cell membranes computed at different experimental time in control and EMF-treated cells. The error bars refer to the Standard Error of the mean NBE values. Cell shapes of control and EMF-treated cells are depicted up and down the graph lines respectively. (B) Normalized Bending Energy values for MDA-MB-231 cell membranes computed at different experimental time in control and EMF-treated cells. The error bars refer to the Standard Error of the mean NBE values. Cell shapes of control and EMF-treated cells are depicted up and down the graph lines respectively.

of the energy that has to be applied in order to produce or modify specific objects [79].

Epithelial cancer cells exhibit high NBE values, while EMF treatment induces a dramatic two-fold reduction on those levels. NBE is inversely correlated with the surface tension, and surface tensions are reflective of intercellular adhesive intensities [80] from which we inferred that EMF-treatment reducing bending energy increases the intercellular adhesion forces. This is noteworthy because surface tension influences both embryonic cell behaviours and metastatic spreading of cancer cells [81,82]. As expected, the bending energy transition experienced by cells under treatment with EMF is associated to a significant reduction in fractal dimension of their cytoplasmic shape (from 1.6 to 1.3). Thus, reversion of tumour shape into a more “physiologic” fractal-dimension implies reduced morphologic instability and increased connectivity between cells. As a consequence of cell shape “normalization”, breast cancer cells exposed to EMF form organized structures (ducts and mammary acini), reactivate signalling pathways and they recover both tight and gap-junctions [83]. In contrast, current cytotoxic anticancer treatments induce a significant increase in cell shape fractal dimension and “may unwittingly contribute to tumour morphologic instability and consequent tissue invasion” [84]. Mild chemotherapeutic regimens do not modify tumour fractal dimension whereas intensive cytotoxic chemotherapy increases fractal values and thus, enhances tissue disorder favouring more malignant phenotypes [85].

$\beta$ -catenin plays critical roles in morphogenesis and in human cancer [86] where it is mainly detectable in the nucleus acting as a transcription factor. In addition, E-cadherin is a negative regulator of  $\beta$ -catenin signalling by stabilising the latter beneath the plasma membrane and thereby sequestering it from the nucleus. Therefore,  $\beta$ -catenin’s functions are strictly linked to its intracellular position and this topological information can be incorporated into fractal analysis by means of the Moran Index (MI) [87]. Confocal microscopy data while correlating spatial  $\beta$ -catenin distribution versus the inner plasma membrane showed a MI ranging from +1 to –1 with 0 corresponding to the absence of a spatial correlation and –1 to maximum dispersion. In EMF-treated epithelial cancer cells,  $\beta$ -catenin distribution was progressively modified evolving from a mean MI value of –0.7 to a mean M.I. value of +0.5 suggesting that, after cell shape normalization,  $\beta$ -catenin moves from the nucleus toward the plasma membrane. Meanwhile, in control cells,

$\beta$ -catenin presents an almost totally disorganized spatial distribution. Concomitantly, in EMF-treated epithelial cancer cells, one can observe a significant increase in E-cadherin release. These results – shape modification and E-cadherin/ $\beta$ -catenin redistribution – collectively suggest that EMF-treated cells undergo a significant transition from a Warburg-like metabolism toward an oxidative pathway. Glycolytic fluxes were reduced with a parallel decrease in lactate, glutathione, glutamine and other compounds. Citrate and de-novo lipogenesis are, in turn, inhibited. In EMF-treated cells, glutaminolysis does not correlate with a simultaneous increase in lactate (as expected when the difference between control and treated cell metabolism should be confined to a mere diversification of energy sources for treated cells), nor with an increase in fatty acid synthesis (as expected when *de novo* cell membrane production is required to sustain cell proliferation). Keeping in mind that proliferation is inhibited in EMF-treated cells, these results suggest that glutaminolysis increases cannot be explained by proliferative needs: this implies that the treated cells devote a higher portion of chemical energy to a *different* anabolic work. Indeed, glutamine is preferentially transformed into proteins and does not appear as lactate.

These data outline how cancer metabolism is driven by the morphogenetic field toward a less dissipative profile. This pattern is mirrored by the changes observed in shape profile. Therefore, fractal dimension emphasizes the neglected link between cell morphology and thermodynamics. According to the Prigogine–Wiame theory of development [88], during carcinogenesis, a living system constitutively deviates from a steady state trajectory that is accompanied by an *increase* in the system dissipation function ( $\Psi$ ) at the expense of coupled processes in other parts of the organism, where  $\Psi = q_0 + q_{gl}$  (meaning, respectively,  $q_0$  oxygen consumption and  $q_{gl}$  glycolysis intensity). NBE represents a “dissipative” form of energy; this effect is mirrored by metabolomic data showing a significant reduction in glycolytic activity (in the presence of unchanged values of oxygen consumption). It follows that in our experimental conditions,  $\Psi$  decreased significantly until a stable state was attained, that is characterized by a minimum in the rate of energy dissipation (principle of minimum energy dissipation) recorded at both morphological and metabolic level of observation [89]. This behaviour is exactly the opposite of what is expected in proliferating cancer cells, as experimentally observed in tumour controls.

## 8. Conclusion

Cell phenotypes represent emergent behaviours that arise through collective interactions among different cellular and microenvironmental components [90]. These behaviours are driven by a non-linear dynamics. Several approaches have been proposed to give a reliable answer to the question, how distinct cell fates emerge? The detailed knowledge of the wiring diagram of the genetic regulatory network and their transcriptome has been insufficient to predict the dynamic landscape of the biological system. On the other hand, modifications of cell shape results in distinct patterns of gene expression [91]. If shape is considered as an intensive system property, i.e., that it requires translating it from a qualitative to a quantitative value by means of fractal analysis, then the behaviour of the system could be represented by a phase-space diagram. Transitions from one phenotype to another are reminiscent of phase transitions observed in physical systems. The description of such transitions could be obtained by a set of morphological, quantitative parameters, like fractal measures. These parameters provide reliable information about *system complexity*. Thereby, phenotypic cell changes could be described in terms of thermodynamic and informational complexity, according to numerous studies revealing strong correlations between shape change and changes in cellular phenotype [92–94].

Within this framework one might ask “how can one perturb the malignant phenotype while bringing it to display a non-malignant behaviour?” The answer to this question positions the old, under-explored idea of differentiation therapy in a new light” [95]. Indeed, an increasing body of experimental results suggests that cancer can be reversed by both physical as well chemical morphogenetic factors belonging to different embryonic morphogenetic fields. These data have contributed to the “rediscovery” of the “morphogenetic field” as a major protagonist in ontogenic and phylogenic change [96]. Indeed, in our view, morphogenetic field effects revert cancer phenotypic traits through the induction of dramatic shape changes [73]. Modification of fractal parameters highlights a parallel change in thermodynamics constraints. Thus, it stands to reason that such modifications might be followed by remarkable changes in cell proliferation patterns, metabolism, as well as tissue differentiating behaviour.

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*Conflicts of interest:* None.

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